CROP ECOLOGY, MANAGEMENT & QUALITY

Butterbean Seed Yield, Color, and Protein Content Are Affected by Photomorphogenesis

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ABSTRACT

Yield and nutrient content of edible beans are important to growers and consumers. Our objective was to determine whether some colors of light reflected to growing bean plants could affect photomorphogenesis enough to result in greater seed yield. Speckled butterbean (*Phaseolus lunatus* L.) was used as the test crop because it is a popular food crop, and the speckled areas contain anthocyanins, which function as antioxidants. The plants were grown in drip-irrigated rows that were covered with plastic mulch to conserve water and to reflect morphogenic light. Black served as the control; and red, green, and white mulch surface colors were used to reflect different quantities of blue (BL), red (R), and far-red (FR) light to the plants from emergence to ripening of seed. Seed yield over red was significantly greater than over black, green, or white. The area of seed coat covered by dark speckles was highest over red and lowest over black and green. Protein concentration on a seed weight basis did not differ among colors, but significantly more seed protein per plant was found in beans that developed over red than over black, green, or white surfaces. We conclude that altering the amounts of BL, R, and FR reflected to developing speckled butterbean plants can alter physiological processes enough to affect seed yield, anthocyanin-containing area on seed coats, and amount of seed protein per plant.

Yield and nutrient content are important characteristics of food crops, and they are influenced by a combination of genetics and the total environment in which the plants are grown. Both photosynthetically active and photomorphogenically active light are critical components of the growth environment. Plant canopy interception and use of photosynthetically active light have been studied for many years (Loomis and Williams, 1963; Monteith, 1965; Hesketh and Baker, 1967). However, management of photomorphogenesis under field conditions is an emerging strategy (Britz and VanDerWoude, 1992; Kasperbauer, 1992, 1999). Morphogenic light is important to plant productivity because it acts through the natural growth regulatory system to influence allocation and use of the products of photosynthesis within growing plants.

Basic studies of effects of photomorphogenesis on various aspects of plant growth, development, and physiological processes have been conducted in controlled environments for more than 40 yr (for example: Parker et al., 1946; Downs et al., 1957; Kasperbauer et al., 1963). More recently, a new approach to food crop production was opened by the discovery that wavelength combinations [especially far-red (FR), red (R), and the FR/R photon ratio] reflected from nearby growing plants, some natural soil colors, and artificially colored materials on the soil surface can act through the natural phytochrome system to influence development and productivity of sun-grown plants (Ballaré et al., 1990; Decoteau et al., 1989; Hunt et al., 1989; Kasperbauer, 1987, 1992, 2000; Kasperbauer and Hunt, 1992, 1998). Use of colored mulches offers a science-based procedure that can alter wavelength distribution in light reflected to sun-grown plants and modify gene expression enough to improve yield and nutrient content of some food crops.

Growing plants over colors of mulch that reflect more R and FR and a higher FR/R photon ratio than are reflected from standard black plastic has resulted in increased yield of perishable food crops such as tomato (*Lycopersicon esculentum* Mill.) (Kasperbauer and Hunt, 1998) and strawberry (*Fragaria ananassa* Duch) (Kasperbauer, 2000). Both of these crops produced higher fruit yield when grown over a red plastic that was formulated to reflect very little blue (BL) and higher R/BL and FR/R photon ratios versus standard black plastic mulch, when other production practices were the same. In addition, compounds that improve nutrient content and flavor of strawberries were increased when grown over the red- verses black-mulched field plots (Kasperbauer et al., 2001; Loughrin and Kasperbauer, 2002). Other colors of light, including blue, reflected to leaves from mulches have affected nutrient content of root crops such as turnip (*Brassica rapa* L.) (Antonious et al., 1996).

We hypothesized that yield of a less perishable food crop, such as edible beans, would also respond to morphogenic light reflected to the plants during seed development. We used speckled butterbean (*Phaseolus lunatus* L.) as the test crop. Our objectives were to determine whether color of light reflected to the developing butterbean plants from materials on the soil surface could affect (i) yield of ripe seed, (ii) protein content, and (iii) seed coat color, which is attributed to content of anthocyanins that are known to function as antioxidants.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

A bush type of speckled butterbean (cv. Jackson Wonder) was used because of its growth habit, local popularity, and the fact that the dark areas on the surface of ripe seeds are due to anthocyanins, which can function as antioxidants in the human diet (Ames et al., 1993; Wang et al., 1997). The plants were grown in drip-irrigated raised-beds of Norfolk loamy sand (Typic Kandiudults) in the Small Farm Research Area of the Coastal Plains Soil, Water and Plant Research Center near Florence, SC, in 1997 and 2001. A randomized

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Abbreviations: BL, blue; FR, far-red; R, red light.

complete block design was used each year of the 2-yr experiment. The raised beds were covered with plastic mulch to conserve water, and different surface colors on the mulch were used to reflect various combinations of BL, R, and FR to the developing plants to determine whether reflected morphogenic light could influence yield and nutrient content. To test the hypothesis that reflected morphogenic light could influence seed yield and some quality characteristics, the plants were spaced farther apart than in most production systems so that light reflected from the colored plastic on the soil surface could reach the developing pods.

Each year, 90-cm-wide by 15-cm-high raised beds were prepared at 1.8-m intervals. Drip irrigation tubes were placed on top of the beds and covered with 1.5-m-wide standard black plastic mulch. There were three such beds each year. Each of them contained four 6-m plots with different colored surfaces (black, red, green, and white) to reflect different combinations of BL, R, and FR to the developing plants. The sequence of color was randomized within each bed, each year. Within each bed, black was obtained by leaving 6 m of the black bed cover exposed. Red was obtained by covering 6 m of the black plastic bed cover with red plastic [Selective Reflective Mulch (SRM-Red), Ken-Bar, Reading, MA] that was formulated to reflect a wavelength combination that favored yield of above ground crops such as tomato and strawberry, as discussed in the introduction of this paper. Exterior enamels were painted onto the black plastic bed covers to provide the green and white surface colors. Measurements and spectral distributions of light reflected from the colors were as described in a previous report (Loughrin and Kasperbauer, 2000). The four colors were used because black reflected less than 6% of any color (it served as the control), red also reflected less than 6% of the BL but greater amounts of R and FR, green reflected about 6% of the BL, less than 10% of the R, and a higher amount of FR (it was used to mimic reflection from green leaves), and white reflected about 40% of all colors (including BL) that impinged on it. Each color used in our study reflected the same spectrum each year.

Seeds were sown in mid-April through 6.5-cm (diam.) holes cut 60 cm apart along the ridge of the plastic-covered raised-beds. The seedlings were thinned to one per hole soon after emergence. Each year there were nine plants per mulch color within each of the three replicate beds. In this system, the holes allowed heat to escape from below the plastic and there was a high probability that the developing pods and nearby leaves received light reflected from the mulch color over which the beans developed.

Seed Yield and Appearance

Plants over all four colors developed ripe seed continuously from late July until frost each year. To keep variables other than reflected light as constant as possible for this study, we compared seed that ripened in August within each year.

In 1997, we removed and discarded the ripe pods present on 29 July and then harvested all pods that ripened between 30 July and 6 August. All pods from the nine plants within each plot (color) were pooled within each of the three beds (reps) and processed for seed per pod and yield. The seed were then stored in darkness at 5°C until amount of speckling was determined and seed protein measured.

In 2001, all ripe pods were harvested on a per plant basis from the first six plants within each color, within each of the three reps on 25 August. As in 1997, all ripe pods were removed in a preliminary harvest (10 August) so that seed yield and quality determinations would be done with newly ripened seed that developed over all colors during the same period. Seed number and weight per plant were determined soon after harvest, and the seed were then stored in darkness at 5°C until the amount of speckling was determined and the seed protein measured.

All seeds that developed over each color in each year were visually examined for the amount of seed surface covered by the dark areas because preliminary observations suggested that the amount and size of speckles were influenced by mulch color over which the seed developed, and that the dark color was due to anthocyanin accumulation. All determinations of the amount of speckling per seed were done by the same person.

Protein Analysis

Seed samples used for analyses developed 10 to 15 cm above the respective colors. Representative samples of seed (about 20 g) that developed over each rep of the variously colored mulches were ground fine enough to pass through a 40-mesh screen, thoroughly mixed, and stored in zipper lock plastic bags in darkness at 5°C until analyzed for protein.

Biuret reagent was prepared in a two-step procedure by first dissolving 1 g KI, 240 mg $CuS0_4$, 3.76 g NaOH, and 24 g NaK tartrate separately in deionized water. The individual solutions were then added to deionized water and the final volume of the reagent was brought to 1.0 L. The solution was stored in darkness at 5°C until used.

One hundred milligrams of seed powder and 20 mg of polyvinyl(poly)pyrrolidone were suspended in 8 mL of 1 *M* NaC1 containing 0.2% (v/v) Tween 20 (polyoxyethylene sorbitan monolaureate 20). The vials were mixed thoroughly and incubated at 50°C for 2 h with periodic mixing. The vials were then centrifuged and 1 mL of solution was added to each cuvette along with 1 mL of biuret reagent. Absorption was read at 550 nm on a UV/Visible spectrophotometer. Protein levels were quantified versus standards of bovine serum albumin (Aldrich Inc., Milwaukee, WI).

Statistical Analysis

Data were analyzed by analysis of variance with PROC ANOVA using the SAS system for Windows, version 6.12 (SAS Institute, 1996).

RESULTS AND DISCUSSION

Seed Yield

Since an objective was to determine whether reflected color combinations that have resulted in increased yield of perishable crops such as tomato and strawberry would also influence yield of a less perishable food crop, we focused on butterbean seed that developed and ripened over standard black (the control treatment) versus colors that reflected various combinations of BL, R, and FR. We determined the weight and number of ripe seeds as well as some characteristics of early crop butterbeans that developed over the different colors in 1997 and 2001 (the experiment was not conducted in 1998 through 2000).

1997

Yield and dry matter distribution between seeds and pods that developed and ripened on plants grown over the different colors in early August of 1997 are summa-

Table 1. Early season yield of ripe butterbeans that grew over different colored mulches in 1997 and ripened during a 1-wk period ending 6 August.

	Mulch color				
Characteristic	Black	Red	Green	White	Significance
	Seed yield per plant				
Seed wt. (g)	12.88 b†	21.51 a	8.93 b	14.57 b	0.021
Seed (no)	53.44 b	84.92 a	37.02 b	57.08 b	0.019
Pod wt. (g)	11.64 b	19.84 a	7.82 b	13.22 ab	0.028
Pods (no)	24.03 b	37.71 a	17.08 b	25.48 b	0.017

† Values are means for three replicates of nine plants each, expressed on a per plant basis. Within each row, means followed by the same letter do not differ significantly at $P = 0.05$.

rized in Table 1. Values for seed weight and number, and for pod number were greatest ($P = 0.05$) over the red mulch.

The numbers of pods with one, two, three, or four seeds were determined over each of the four colors. While the number of seed and the number of pods were both greatest over red (see Table 1), there were no significant differences among colors for percentages of the pods with only one seed. The same held true for percentages of pods with two seeds and for those with three seeds. However, the percentage of pods with four seeds was significantly (at $P = 0.05$) greater over red than over black, green, or white (data not shown). Clearly, there were some differences in seed yield and seed per pod for beans grown over the four colors in 1997.

2001

Yield and dry matter distribution between seeds and pods that developed and ripened over the same four colors during a 2-wk period in August of 2001 are summarized in Table 2. Data were collected on an individual plant basis from the first six plants within each color from each rep, and means for the 18 plants per color are presented. As occurred in 1997, the number and weight of seeds and the weight of pods that developed over the red mulch were greater ($P = 0.05$) than those that developed over the other colors. Seed numbers and weights per plant were lowest over the black and green. Seed yield over white was intermediate between those that developed over red versus black.

Although number of seed per pod were not determined in 2001, the facts that (i) average weight per seed did not differ significantly among colors and (ii) the ratio of ripe seed weight to dry pod weight was highest over the red mulch suggest that there was a greater number of seed per pod over red. This interpretation is consistent with the actual counts taken in 1997.

Our results suggest that the low amount of BL coupled with the higher R/BL and FR/R photon ratios reflected from the red influenced allocation of relatively more photosynthate to the edible beans. This is consistent with the theoretical basis for photomorphogenic action of light reflected from the red mulch (Kasperbauer, 1992, 1999), and with yield increases previously found with perishable food crops such as tomato and strawberry grown over red versus standard black mulches (as discussed in the introduction of this report).

Table 2. Early season yield of ripe butterbeans that grew over different colored mulches in 2001 and ripened during a 2-wk period ending 25 August.

	Mulch color				
Characteristic	Black	Red	Green	White	Significance
	Seed yield per plant				
Seed wt. (g)	65.6 c†	125.1 a	72.4 c	97.0 b	<0.0001
Seed (no.)	211.9 c	385.2 a	234.6 c	316.9 b	0.0005
Pod wt. (g)	37.7 c	66.2 a	44.2 c	55.4 b	0.0005
	Weight/seed and seed/pod wt. ratio				
Weight (mg)/seed	310 a	325 a	309 a	306 a	NS
Seed/pod (wt. ratio)	1.74 bc	1.89 a	1.64 c	1.75 bc	<0.0001

† Values are means for 18 plants (six plants per each of three replicates per color). Within each row, means followed by the same letter do not differ significantly at $P = 0.05$.

Seed Color

While separating seed from pods in 1997, it became apparent that the amount and size of speckles (dark areas) on the otherwise light-colored seed coats differed among beans grown over some colors of mulch. Preliminary analyses of extracted dark pigments showed that they were anthocyanins, and anthocyanins are known to function as antioxidants in the human diet (Wang et al., 1997). The ripe beans were visually sorted according to the approximate percentage of seed surface that contained the dark-pigmented areas. Data from both years are summarized in Table 3. Seeds that developed over red had greater amounts of dark areas than those that developed over black and green. The intensity of dark pigmentation also varied among seeds (data not shown). More seed with very dark areas were observed among beans that developed over red versus black and green.

Although extraction and detailed analysis of pigments from the dark colored areas were beyond the scope of the present study, observations of the amount of speckles (see Table 3) and the intensity of the dark colored areas suggest greater accumulation of anthocyanins in the speckled areas of butterbeans that developed and ripened over the red than over black and green surfaces both years. Additional work on the affect of R/BL ratio on thickness of pod walls and this on possible transmission of R and FR through pod walls to developing seeds may help explain differences in amount of pigment accumulation in seed coats.

Protein

Legume seeds are a major source of protein in the human diet. Therefore, it was important to determine whether color of light reflected from the soil surface to developing butterbean plants could influence protein content of the ripe seed. In 1997, no significant differences in milligrams of protein per gram of seed were

Table 3. Percentages of seeds with more than about 75% of the surface covered with dark pigmented areas in 1997 and 2001.

	Mulch color			
Year	Black	Red	Green	White
1997	16.1 b†	23.5 a	15.3 b	17.4 ab
2001	20.5 b	26.3 a	14.6 c	22.1 ab

† Within each row, values followed by the same letter do not differ significantly at $P = 0.05$.

Table 4. Protein concentrations in butterbean seed that developed and ripened over different colored mulches in 2001.

	Mulch color			
Seed Protein	**Black**	**Red**	**Green**	**White**
mg/g of seed	**304 a†**	**298 a**	**307 a**	**293 a**
g/plant	**19.9 c**	**37.2 a**	**22.2 c**	**28.4 b**

† Within each row, values followed by the same letter do not differ significantly at $P = 0.05$.

observed among the four colors (data not shown). However, more grams of seed were found on plants grown over the red than over the other colors (see Table 1). Protein concentrations of butterbeans that developed and ripened above the different mulch colors in 2001 are shown in Table 4. The data are presented in two ways: (i) as milligrams of protein per gram of the ripe seed, and (ii) as grams of seed protein per plant, which might be important in areas in which growers with very limited space desire to maximize output per plant. As shown in Table 4, seeds that developed over the four colors did not differ significantly ($P = 0.05$) in concentration of protein on a milligram per gram basis. However, when seed protein content was expressed as grams per plant, the highest values were evident in beans that developed over the red mulch because plants growing over red yielded more grams of beans per plant. Thus, the greatest yield of dry beans and the greatest amount of protein per plant occurred over the red surfaces, and the lowest amounts per plant occurred on plants growing over the black and green surfaces. The important point here is that the ripe seed yield response to reflected color by this pod-borne food crop is consistent with yield response of the more perishable tomato and strawberry fruit. An added bonus appears to be a greater concentration of anthocyanin and the greater yield of seed protein per plant over the red reflecting surfaces.

SUMMARY

Occurrence of the greatest butterbean yield over the red versus black mulch is consistent with the hypothesis (see Kasperbauer, 1992, 1999) that a low amount of BL coupled with higher amounts of R and FR, and a higher FR/R photon ratio reflected to plants growing in sunlight would act through the natural phytochrome system and result in a greater amount of photoassimilate allocated to above-ground portions of the plant, including seed. Increased butterbean seed yield over the red mulch is also consistent with previously reported increased yield of perishable crops such as tomato and strawberry over red versus black surfaces. However, the present discovery with edible ripe beans may be of even greater worldwide importance because they are a less perishable food crop that can be stored under less precise conditions before being consumed. We postulate that awareness of plant responses to color of reflected light under field conditions can become increasingly important in development of production systems for food and specialty crops grown for both yield and concentrations of compounds that contribute to human nutrition and health.

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